only the sum of the rate coefficients can be measured for a two state system, maximum likelihood methods allow the determination of the individual rate coefficients, and therefore also the equilibrium constant. Here we apply a maximum likelihood method recently developed by Gopich and Szabo (*J.Phys.Chem.*113pp10965-10973(2009)) to the photon-by-photon trajectories of a two-state designed protein, α_3D, that folds in ~ 1ms, too fast to measure accurately from waiting time distributions in FRET trajectories. Experiments were carried out on both freely-diffusing and immobilized molecules. The FRET efficiency distributions in the free diffusion experiment are broadened by the folding/unfolding transitions occurring within the time bin, a phenomenon similar to line broadening in NMR experiments. Mean FRET efficiencies and rate coefficients extracted using the Gopich-Szabo method were found to be reliable by comparing the sum of the rates with the relaxation rates obtained from the donor-acceptor cross correlation function. Finally, photon trajectories can be divided into folded and unfolded segments at a single photon level using the hidden Markov model (Viterbi algorithm) with extracted parameters.

158-Pos

Conformations and Dynamics of Polypeptide Chains Revealed By Tryptophan-Cysteine Contact Formation Kinetics

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Spectroscopic probes sensitive to intra-chain contact formation events in polypeptides are increasingly used to study the conformational and dynamical properties of different amino acid sequences. Quenching of the triplet state of tryptophan by close contact with cysteine enables the measure of contact formation rates without the need of extrinsic probes, thus being suitable for the study of natural proteins and peptides. We illustrate the use of this method to investigate the unfolded state of small proteins in conditions close to native and the kinetics of weakly structured protein fragments. The coexistence of different conformational states can be revealed from the non-exponential relaxation of the excited triplet, enabling the characterization of both the chain dynamics for each state and the transition kinetics. Moreover, the rate of contact formation measured for the least structured states is compared with those observed for model disordered peptides, allowing to estimate the strength of electrostatic and hydrophobic interactions between residues other than the probes. We test this approach with the widely studied GB1 15-residue C-terminal, which folds into a beta-hairpin structure. The kinetics of elementary conformational steps leading to the folded state is outlined, revealing the presence of misfolded states as proposed in recent computational works.

159-Pos

Electrostatic Interactions Affect the Mechanical Stability of Elastomeric Proteins

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It was predicted that the mechanical stability of elastomeric proteins can be affected by electrostatic interactions (Craig et al, Structure, 12, 2004, 21). To directly test this prediction, we engineered a bi-histidine mutant of a small protein GB1 (biHis-GB1). The two histidines were engineered across two force-bearing beta strands. Histidine residues can exist as protonated or deprotonated states depending on pH, thus we can adjust the pH value of the solution to modulate the electrostatic interactions between the two engineered histidine residues. We used single molecule atomic force microscopy to directly measure the effect of electrostatic interactions on the mechanical stability of biHis-GB1. We found that the unfolding force of biHis-GB1 gradually decreases as the electrostatic repulsion increases due to the lowering of pH value from 8.5 to 4. This result suggested that electrostatic interactions can indeed affect the mechanical resistance of biHis-GB1. We anticipate that this effect can be utilized as an effective method to tune the mechanical stability of elastomeric proteins at the single molecule level.

160-Pos

Molecular Mechanism of Urea-Induced Protein Denaturation

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For more than half a century, urea has been used as a strong denaturant in protein folding/unfolding studies. However, the molecular mechanisms of urea-induced protein unfolding still remain unclear. This lack of understanding is to some extent reflects the scarcity of direct thermodynamic information that can be used to characterize interactions of urea with amino acid side chains and the peptide group. We recently demonstrated that volumetric measurements combined with statistical thermodynamic approach may represent a novel and effective way to tackle this problem [Lee, S. & Chalikian, T. V. (2009) J. Phys. Chem. B. 113, 2443-2450]. In this work, we employ high precision acoustic and densimetric techniques to quantify the solvation properties of solutes in the presence of urea. Specifically, we report the partial molar volumes, V°, and adiabatic compressibilities, K_S°, of *N*-acetyl amino acid amides containing all 20 naturally existing amino acid side chains and oligoglycines, $(Gly)_{1\text{-}5}$, at urea concentrations ranging from 0 to 8 M. Using our developed statistical thermodynamic approach, that links volumetric observables of a solute with solute-solvent and solute-cosolvent interactions in binary solvents, we evaluate the binding constants, k, and elementary changes in volume, ΔV, and compressibility, ΔK_S, accompanying the replacement of water in the vicinity of the solutes with a urea molecule. While the binding constants are essentially similar for all protein groups, the magnitude and the sign of the determined values of ΔV and ΔK_S vary markedly. The latter values reflect the nature of urea interactions with specific functional groups and the concomitant changes in hydration. In general, our results are consistent with a picture in which urea interacts with polar, non-polar and charged groups with comparable affinities, although the underlying forces stabilizing each type of interaction depend on the chemical nature of the interacting group.

161-Pos

Confined Dynamics of a Ribosome-Bound Nascent Globin: Cone Angle Analysis of Fluorescence Depolarization Decays in the Presence of Two Local Motions

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We still know very little about how proteins achieve their native three-dimensional structure in vitro and in the cell. Folding studies as proteins emerge from the mega Dalton-sized ribosome pose special challenges due to the large size and complicated nature of the ribosome-nascent chain complex. This work introduces a combination of three-component analysis of fluorescence depolarization decays (including the presence of two local motions) and in-cone analysis of diffusive local dynamics to investigate the spatial constraints experienced by a protein emerging from the ribosomal tunnel. We focus on E. coli ribosomes and an all-alpha-helical nascent globin in the presence and absence of the chaperones DnaK and trigger factor. The data provide insights on the dynamic nature and structural plasticity of ribosome-nascent chain complexes. We find that the sub-ns motions of the N-terminal fluorophore, reporting on the globin dynamics close to the N terminus, are highly constrained both inside and outside the ribosomal tunnel, resulting in high-order parameters (>0.85) and small cone semiangles ($<30^\circ$). The shorter globin chains buried inside the tunnel are less spatially constrained than those of a reference sequence from a natively unfolded protein, suggesting either that the two nascent chain sequences have a different secondary structure and therefore sample different regions of the tunnel or that the tunnel undergoes local structural adjustments to accommodate the globin sequence. Longer globins emerging out of the ribosomal tunnel are also found to have highly spatially constrained slow (ns) motions. There are no observable spectroscopic changes in the absence of bound chaperones. The data presented here show that the ribosome plays an active role in cotranslational folding and it influences the dynamics and conformation, of nascent polypeptides and proteins.

162-Pos

Unraveling the Possible Mechanism Behind Leptomeningeal Amyloidosis Using as Model a Highly Unstable Transthyretin Tetramer

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Transthyretin (TTR) is a 127-residue -βsheet-rich protein homotetrameric that transports thyroxine in the blood and cerebrospinal fluid (CSF). Among all TTR variants, A25T is the most unstable tetramer. Its great instability induces TTR degradation in the endoplasmic reticulum of the hepatocytes, while thyroxine (T4, a natural ligand of TTR) leads to A25T secretion in the CSF by the choroid plexus. In the present study we aimed to determine the structure of A25T by X-ray crystallography in the apo form and in complex with T4. Also, by using high hydrostatic pressure, we have showed that the tetramers of A25T were less stable than the wt and L55P (the most aggressive variant of TTR). Besides, A25T showed to be the most amyloidogenic variant thus far investigated, aggregating in conditions where wt and L55P remain mostly soluble. Using HPLC and native PAGE, we monitored acrylodan-labeled TTR aggregation in the human plasma. The aggregates formed displayed the typical amyloid structure. In the presence of monomers of T119M, a non-amyloidogenic variant, aggregation of A25T was remarkably reduced, pointing to the use of T119M monomers as a strategy to avoid TTR aggregation. The crystal structure of A25T, when compared to that of the wt protein, shaded light into the